

# The Role of 'Paraoxonase-1 Activity' as an Antioxidant in Coronary Artery Diseases

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## ABSTRACT

**Background and Objectives:** A majority of the Coronary Artery Diseases (CAD) result from complications of atherosclerosis. There is a growing body of evidence which has revealed that the reduced activity of the HDL-associated enzyme, paraoxonase1 (PON1), is predictive of vascular disease in humans, which include the results from prospective studies. The mechanisms by which PON1 activity influences risk of vascular disease continue to be evaluated. It is generally thought that PON1 contributes to the antioxidant, and thus, to the antiatherogenic properties of High Density Lipoproteins (HDL). Depleted antioxidant levels could be a risk factor for coronary artery disease. Hence, this study was done to evaluate PON1, as antioxidant, in CAD patients.

**Methods:** This study was done to determine serum levels of PON1 activity in 50 controls and in 60 clinically and ECG proven CAD cases and to compare PON1 activity with total cholesterol and triglycerides.

**Results:** Serum levels of PON1 activity ( $p < 0.001$ ) were significantly lower in CAD cases than in controls. Serum total cholesterol ( $p < 0.001$ ) and triglyceride ( $p < 0.001$ ) levels were significantly higher in CAD cases than in controls. There was a negative correlation between PON 1 activity and total cholesterol and triglycerides. The negative correlation between PON1 activity and total cholesterol was significant ( $p < 0.05$ ).

**Interpretation and Conclusion:** From our present study, we can conclude that PON1 can exert a protective effect on HDL by preventing its oxidative damage. Further, a decreased PON 1 activity may be a risk factor for CAD, which is likely to be explained by derangement of PON 1 activity towards lipid peroxidation. This study suggested that serum antioxidant activity of PON1 was an important factor which provided protection from oxidative stress and lipid peroxidation in CAD. Thus, evaluating the effects of PON 1 for CAD patients may be promising in the treatment and prognosis of CAD.

**Key words:** Antioxidants, Coronary artery disease, Oxidative stress, Paraoxonase1

## INTRODUCTION

Diseases of the coronary arteries are almost always caused by atheroma (atherosclerotic plaque) and its complications, particularly, thrombosis. Endothelial dysfunction and lipid peroxidation have been observed in patients with established coronary artery disease or coronary risk factors, both in the coronary and peripheral vasculature [1,2].

Oxidative modification of Low Density Lipoproteins (LDLs) in the artery wall is now widely being considered to play an important role in the development of atherosclerosis. Binding of LDL to proteoglycans traps LDL in the arterial intima, which provides the opportunity for oxidation of LDL lipids. This mildly oxidised LDL can induce endothelial cells to produce chemoattractants, adhesion molecules, and colony stimulating factors for monocytes. Adhesion to the vascular endothelium, followed by transmigration of the circulating monocytes into the arterial intima, are early steps in the formation of atherosclerotic lesions. HDL inhibits LDL oxidation by inhibiting the biological effect of mildly oxidised LDL on the monocyte endothelial cell interaction. These properties of HDL may contribute to the inverse association between plasma HDL levels and risk of developing coronary artery disease, which has been observed in epidemiological studies [3].

Paraoxonase (EC.3.1.8.1, arylalkylphosphatase) is an esterase enzyme that is synthesized by the liver and it is associated with

HDL in the blood. There is considerable evidence to prove the fact that the antioxidant activity of HDL is largely due to the PON1 which is located on it [4]. The enzyme hydrolyzes aromatic carboxylic acid esters, organophosphates and oxidised phospholipids. Hydrolysis of oxidised phospholipids by paraoxonase destroys the biologically active lipids in mildly oxidised LDL. Virtually all of PON1's activity is associated with HDL-cholesterol. PON1 appears to prevent both LDL and HDL from oxidation. Also, it has been shown that mice which lack serum PON1 activity are susceptible to atherosclerosis [5,6].

Hence, the present study is undertaken to evaluate the role of PON1 as an antioxidant in CAD and to correlate this antioxidant with the concentration of serum total cholesterol and triglycerides in CAD.

## METHODOLOGY

A cross sectional study was carried out for a period of one year. Based on the inclusion and exclusion criteria, a total number of 60 CAD cases and 50 age and sex matched controls were selected for the present study. Patients were consecutively selected as and when they presented to us. Both cases and controls were interviewed to obtain relevant data. Informed consents were obtained from all the subjects who were involved in the study. This study was approved by the ethical and research committee. The patients and controls voluntarily participated in the study.

**Inclusion Criteria:** Cases were clinically and ECG proven CAD patients with and without complications. Controls were healthy individuals, who were age and sex matched, without any major illnesses.

**Exclusion Criteria:** The CAD patients who had liver diseases, renal diseases and thyroid diseases and who were on antioxidant supplementation.

About 5 ml of plain blood was drawn under aseptic precautions from a large peripheral vein. The blood was allowed to clot for about 30 minutes and then, serum was separated by centrifugation at 5000 rpm. It was kept at 4°C until further analysis was carried out. Biochemical parameters which were estimated in the serum samples were:

1. Arylesterase activity of PON1 by a spectrophotometric method by using *p*-nitro phenylacetate as the substrate [7]. PON specificity towards endogenous serum and tissue substrates was not well-characterized, and therefore synthetic substrates are used to monitor the enzyme's activity.
2. Total cholesterol by the enzymatic CHOD/PAP method [8].
3. Triglycerides by the GPO/PAP method [9].

Student's 't' test, followed by Pearson's correlation co-efficient, was employed for the statistical analysis of the data, to compare various parameters in the study.

## RESULTS

A total number of 110 subjects were included in this study. Among them, 50 were controls who were normal healthy individuals and 60 were coronary artery disease patients (cases). Among cases, the maximum number of CAD patients was in the age group of 40 to 70 years. Among the controls, 29 were men and 21 were women and their mean age was 50.0 ± 15.5 years. Among cases, 40 were men and 20 were women and their mean age was 57.2 ± 13.0 years.

The mean serum level of PON1 activity was in the range of 22.0 ± 3.4 nmol/ml/min in cases and it was in the range of 50.9 ± 8.4 nmol/ml/min in controls. The mean serum levels of total cholesterol and triglycerides in cases were in the ranges of 222.6 ± 49.5mg/

dl and 206.1 ± 57.6mg/dl respectively and in controls, they were in the ranges of 154.5 ± 32.7 mg/dl and 123.0 ± 32.6 mg/dl respectively.

Serum levels of PON1 activity ( $p < 0.001$ ) were significantly lower in CAD cases than in controls. Serum total cholesterol ( $p < 0.001$ ) and triglyceride ( $p < 0.001$ ) levels were significantly higher in CAD cases than in controls [Table/Fig-1].

There was a negative correlation between PON 1 activity and total cholesterol and triglycerides. The negative correlation between PON1 activity and total cholesterol was significant ( $p < 0.05$ ). Age had a positive correlation with total cholesterol and triglycerides in both cases and controls, which was not significant [Table/Fig-2].

## DISCUSSION

PON 1 is a calcium dependent esterase which is closely associated with HDL, which contains apo A I. PON 1 has been reported to contribute to the antioxidant properties of HDL by decreasing the accumulation of lipid peroxidation products on LDL, by hydrolyzing lipid peroxides in the lipoprotein. As an antiatherogenic mediator, HDL, other than playing an important role in the reverse cholesterol transport, protects LDL against oxidation. This effect of HDL of decreasing LDL peroxidation is maintained for a longer time than that of antioxidant vitamins and it could thus be more protective. The inhibition of LDL oxidation by HDL has been attributed largely to the HDL bound enzyme, PON1.

In our present study, serum PON 1 levels were significantly decreased ( $p < 0.001$ ) in CAD cases than in controls. These findings were in accordance with the earlier reports of Tward A et al., [6], Graner M et al., [10], James RW et al., [11], Senti M et al., [12], Nabatchian F et al., [13], Michael NO et al., [14] and Sarkar PD et al., [15]. In our study, serum levels of PON 1 were negatively correlated with total cholesterol and triglycerides. This was in accordance with the studies which were conducted by Rozek L.S et al., [5]. and Graner M et al., [10].

Oxidative modification of LDL is believed to be central to the initiation and progression of atherosclerosis. LDL is believed to exit the lumen of arteries and become trapped in the subendothelial space, perhaps by the binding of apo B-100 to intimal

	No. of subjects	PON1 activity nmol/ml/min (Mean ± SD) (Range)	Total cholesterol mg/dl (Mean ± SD) (Range)	Triglycerides mg/dl (Mean ± SD) (Range)
Cases	60	22.0 ± 3.4	222.6 ± 49.5	206.1 ± 57.6
Male/Female	40/20	(16.7 to 33.5)	(102 to 309)	(76 to 33)
Controls Male / Female	50	50.9 ± 8.4	154.5 ± 32.7	123.0 ± 32.6
	29/21	(29.5 to 67.0)	(89 to 216)	(67 to 186)
Mean diff.		28.9 ↓	71.5 ↑	83.1 ↑
t-value*		t = 22.9	t = 8.63	t = 9.50
p-value		< 0.001	< 0.001	< 0.001

**[Table/Fig-1]:** Comparison of Serum PON1 activity, total cholesterol and triglycerides in the serum of CAD cases and controls

\*Student 't' test

Correlation between	Cases				Controls			
	Total cholesterol		Triglycerides		Total cholesterol		Triglycerides	
	r	p	r	p	r	p	r	p
Age	+0.12	0.35	0.03	0.81	0.09	0.53	0.09	0.52
PON 1	-0.32	< 0.05 (S)	-0.23	0.08	0.05	0.74	0.28	0.06

**[Table/Fig-2]:** Relationship between serum PON1 activity, total cholesterol and triglycerides in the serum of CAD cases and controls

r – Pearson's correlation co-efficient

– sign indicates negative (inverse) correlation

+ sign indicates positive correlation

proteoglycans. Once it is trapped, LDL may become oxidised directly by the cellular byproducts of respiration or enzymatically by lipoxygenases, myeloperoxidases, NADPH oxidase, cytochrome P-450, and others [6]. HDL exerts an antiatherogenic effect by inhibiting LDL from oxidation. The inhibitory effect of HDL on LDL oxidation is brought about by HDL associated enzymes – PON, Lecithin Cholesterol Acyl Transferase (LCAT) and platelet activating factor acyl hydrolase (PAF – AH). PON hydrolyzes the oxidised phospholipids of oxidised LDL and it also prevents the activation of monocytes by oxidised LDL. The inhibitory effect of PON on LDL oxidation includes the inhibition of initiation (conjugated dienes formation), propagation (peroxide formation) and decomposition (aldehyde formation) phases of LDL [16].

In addition to preventing LDL oxidation, PON 1 may preserve other functions of HDL, such as reverse cholesterol transport by reducing the oxidative damage to HDL. This effect could be related to the ability of PON in hydrolyzing lipoprotein associated peroxides. PON is an aryl dialkyl phosphatase (esterase) which is capable of hydrolyzing the O-P ester bond in paraoxon. A similar type of bond may exist in lipoprotein associated phospholipid peroxides and in cholesteryl ester peroxides. PON reduces cholesteryl linoleate hydroperoxide levels in oxidised HDL, and it produces cholesteryl linoleate hydroxides, but not fatty acid hydroperoxides [16].

Under oxidative stress, not only LDL is susceptible to lipid peroxidation, but all other serum lipids, which include HDL, are also prone to oxidation. In fact, HDL has been shown to be the major carrier of lipid hydroperoxides in human serum. HDL associated cholesteryl ester hydroperoxides are more rapidly reduced to their less reactive hydroxides than those which are associated with LDL [17]. Oxidative modification of HDL has also been shown to impair the ability of the lipoprotein in promoting a cholesterol efflux. Thus, inhibition of HDL oxidation by PON may preserve the antiatherogenic functions of HDL in reverse cholesterol transport, as well as its protection of LDL from oxidation [18,19].

Marit Graner et al., [10] reported that PON 1 activity was a significant determinant of the severity of CAD, which was independent of HDL cholesterol. Conversely, Navab et al., [19] reported a failure of HDL in protecting LDL from oxidation in CAD patients, which they proposed, was due to low serum PON activity despite relatively normal HDL concentration.

Reduced serum PON 1 activity in CAD is possibly caused by increased oxidative stress. PON 1 activity has been shown to be reduced in the course of oxidative incubation with Cu<sup>2+</sup> induced peroxidation of LDL. Oxidised LDL appears to inactivate PON 1 through interactions between the enzyme free sulfhydryl groups and oxidised lipids, which are formed during LDL oxidation [20]. This leads to the inability of PON 1 in inhibiting LDL oxidation and in preventing HDL from further oxidation, which results in an inability of HDL in inducing a cellular cholesterol efflux from macrophages. The possible mechanisms for PON inactivation during LDL oxidation may be copper ion binding to PON, free radicals attack on PON and/or effect of lipoprotein associated peroxides.

Several epidemiological studies have also suggested that decreased PON 1 activity is an independent risk factor for the development of CAD [12,13,15,20,21].

## CONCLUSION

Indirect evidence of the effects of free radicals in coronary artery disease status may be obtained by comparing antioxidant

concentrations, because serious damage by free radicals implies an insufficiency of the body's multilevel defense systems against free radicals. The present study demonstrated that HDL associated PON1 can exert a protective effect on HDL functions. This effect is due to PON's peroxidase – like activity and it contributes to HDL's antiatherogenic properties. Hence, evaluating the effects of PON 1 for CAD patients may be promising in the treatment and prognosis of CAD. PON1 would thus seem worthy of further studies as an aetiological factor in the development of CAD and perhaps other diseases.

## LIMITATIONS

The study population was relatively small and hence, care should be exercised in the extrapolation of the findings to other populations. The magnitude of interassay variation in the PON1 measurements was not sufficiently large to account for the difference in PON1 activities. Although PON1 activity and concentration were determined genetically, various factors such as diet, lifestyle and environmental factors can influence PON1 activity and concentration. Degraded cooking oil has been reported to lower serum PON1 levels in humans [22]. Dietary polyphenol increases PON1 activity, as does moderate alcohol intake [23,24]. Hence, additional information is required, particularly about nutritional and pharmacological effects on serum PON1 activity, that might lead to intervention trials, for testing its capacity in preventing atheroma. Information from prospective cohort studies may also be valuable, as would a more detailed knowledge of the basic biochemistry of PON1 action and its interrelations with other HDL enzymes.

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